The Biological Testing Branch is the successor to the Screening Section (SC), Drug Evaluation Branch (DEB), both of which were disbanded as a result of a reorganization in 1986. The Biological Testing Branch assumed the responsibilities of the Screening Section , Drug Evaluation Branch which included the assignment of materials to the testing laboratories, evaluation of the testing of the assigned materials, monitoring the contract(s) performing the testing, and the creation of new tumor test protocols when necessary. The following is a synopsis of coding, testing protocols, methodology, and history of the antineoplastic testing program as conducted by the SC, DEB and the Biological Testing Branch.

Codes utilized on computer generated screening data summaries for testing conducted prior to and including 1986 and changes made on screening data summary codes after 1986 are noted at the appropriate topic. Summaries, for testing based upon the scheme of in vitro testing followed by in vivo testing, are not printed in the above format and data is printed without computer codes, with the exception of the tumor system protocol, which is defined in the tumor code protocol.

In vivo tumor system codes consist of 5 data fields on the screening data summary. The first space indicates the host (3 is mouse, 5 is rat, 7 is hamster,8 is egg, and 9 is tissue culture); the second and third fields designate the tumor line; the fourth field designates the mode of evaluation;; the fifth field designates the site of the tumor implant. Screening data summaries for in vivo testing, for the period from 1986 until the new screening data format, have the mouse strain code in place of the general mouse code of "3". With the exception of the mice in which tumors rose spontaneously, mice used in the experiments were approximately 6 weeks of age. They were received at the laboratories at approximately 5 weeks of age and then held for one (1) week in quarantine before use. Young mice were used to minimize the possibility of an immune response. Mice of both sexes were used, but not in the same experiment (control). Where the tumor might be hormone dependent, the appropriate sex was used. Male mice weighed a minimum of 18 grams and female mice weighed a minimum of 17 gram and only one sex was used in an experiment (control). At each laboratory, every experiment was assigned a number and this number was referred to as the "control number". All mice, at the start of an experiment, weighed within a three (3) gram range. The number of animals, used at each test dose level in an experiment, was usually 6, but in some tumor systems this number was 10. Where there is no listing for the number of animals used per test dose, no verification of the number used could be found and the number should be assumed to be 6. Rats, used in an experiment, were also held for one (1) week in quarantine and weighed between 90 and 110 grams. However, the weights for the rats in the Murphy-Sturm lymphosarcoma testing, (5MS16) were 45-55 grams, and for the walker 256 (5WA_ _) testing they were 50-70 grams. Both sexes were used, but not in the same experiment (control), during the period from the start of the program until the mid 1960's, testing was conducted in the in vivo sarcoma 180, carcinoma 755 and l1210 leukemia tumor systems and the in vitro KB system. In vivo testing was conducted at only one (1) dose per experiment rather than in a dose response. The parameters for efficacy are listed in the published protocols listed below.
In vitro testing is coded with a 9, followed by the tumor line designation code and the number "5". Cytotoxicity is reported as ED50 (estimated dose that was lethal to 50% of the cells) in micrograms per ml.

Listed amongst the vehicles is the code "0M" which is defined as *Klucel*. *Klucel* is hydroxypropyl cellulose, which is synthesized by *Hercules Incorporated,* Wilmington, Delaware. *Klucel* was substituted for methyl cellulose (MC), which was reported to be carcinogenic as we used it, and for carboxymethyl cellulose (CMC), which was found to be toxic in certain treatment schedules as a suspending agent. *Klucel* HF was donated to the National Cancer Institute by *Hercules Incorporated.* For this donated sample, production was halted prior to the addition of silicon dioxide to hydroxypropyl cellulose , and the donated quantity removed was sent to the National Cancer Institute. *Hercules Inc.* adds silicon dioxide to *Klucel* to prevent the "caking" of the powder.
Under separate contracts, a 0.3% solution of *Klucel* in sterile saline was prepared, bottled, and sent to the National Cancer Institute. *Klucel* is solubilized as follows: powder is added to the vortex of well agitated water at room temperature. The rate of addition must be slow enough to permit the particles to separate in the water. Addition of the powder should, however, be completed before any appreciable viscosity build-up is obtained in the solution. The rate of agitation may then be reduced, but continued until a gel-free solution is obtained. Throughout the mixing period the solution temperature should be maintained below 35 C. The viscosity in Centipoise at shear rates as measured by a Ferranti-Shirley instrument are as follows:

Shear Rate	185	1000	1850	10,000	18,500
Viscosity in Centipoise:	7.1	4.2	3.9	2.9	2.5

The rational for the use of silicon dioxide free hydroxypropyl cellulose was the fear of the silicon dioxide precipitating from the solution upon standing.

During the period from the conception of the "Cancer Chemotherapy National Service Center" (CCNSC) - the name was discontinued when the CCNSC program was incorporated into the National Cancer Institute intramural program in the 1960's- until 1986, animal tumor protocols were continually developed and substituted for existing tumor systems in the program. Protocol instructions were sent to the contract laboratories conducting the testing and FOUR (4) publications were printed listing in detail the tumor protocols in
mode as of that date. The FOUR (4) publications are listed below:
Cancer Chemotherapy Reports #1, pages 42-64, 1959
Cancer Chemotherapy Reports #25. 1962.
Cancer Chemotherapy Reports, Part 3, Vol. 3, #2, September 1972
In Vivo Cancer Models, 1976-1982, NIH Publication No. 84-2635, February 1984
The "NSC' notation before the number assigned to a material being tested for antineoplastic efficacy, e.g. NSC749, stands for "National Service Center". Materials were assigned numbers in sequence as they were accepted into the program for testing,.
As noted above, tumor protocols were constantly being modified and developed. For example, with L1210 initially the treatment was intraperitoneal from day 1 post tumor implant until death. This schedule was then changed to from day 1 to day 15, then from day 1 to day 9, and finally days 1-5. The treatment schedule that appears in the table is the last treatment schedule used. In addition, treatment schedules were scheduled day 1 only, days 1,5,9, oral administration, intravenous injection, etc. to determine the optimal route and schedule of an efficacious agent.
In defining a tumor code, an NSC number may appear after the "/". This designates the material to which the tumor is either partially or completely resistant. E.g. 3MP21 is "decoded" as L1210/NSC 755. This is defined as L1210 leukemia resistant to NSC 755.
Resistant tumor lines are developed as follows: Three cages of mice are assigned each tumor generation. The animals in one cage are the untreated control inoculated with the sensitive tumor; the animals in the second cage are inoculated with the sensitive tumor and receive the material at the optimum dose level for that regimen; the mice in the third cage are inoculated with the sensitive tumor and receive the material at a dose level that is not the optimum dose. When necessary to transfer the tumor, the same cages are set up with the animals in the third cage being inoculated with the tumor from the third cage of the previous tumor generation rather than with the "sensitive" tumor, and the dose of the material is elevated. The animals in the first two cages are inoculated with the sensitive tumor. This scheme is followed until there is no significant difference in the time of death between the animals in the untreated, sensitive cage when compared with the animals in the third cage. The animals in the second cage, that are treated with the agent at the optimum dose, are a check that the sensitive tumor is growing normally, and act as a monitor of the efficacy of the material against the sensitive tumor. It is essential to challenge the "resistant" cell line with the agent to which it is resistant each generation. Otherwise, resistance to the agent may be lost after several generations. This occurs with L1210 made resistant to NSC 740, Methotrexate.

Evaluations were either mean tumor weight, median tumor weight, mean survival time (st), or median survival time (st). Where there are "blanks" for information on tumor systems, the original reference was the "Cancer Chemotherapy Reports, vol. 3, no. 2, September 1972) appendix II, which listed the laboratory that conducted this testing. When in vivo murine, rat, etc. screening was terminated in the mid 1980's in favor of an in vitro screen followed by xenograft in vivo tumor model testing, the contracts with the laboratories listed were terminated. and the testing protocols could not be recovered in 1997.
Efficacy, for materials being tested in the in vivo screen, is expressed as a % T/C value. That is, the mean/median tumor weight or the mean/median survival time of the treated group is divided by that of the untreated control group and multiplied by 100.

TESTING WHERE THE HOST WAS AN EGG

This testing was conducted prior to March 5, 1965, at which time screening laboratories were instructed to cease testing in this model. The code for this testing was 8H112, Human sarcoma (HS1). The protocol was published in Cancer Chemotherapy Reports, vol. 25, page 21, 1962.

IN VITRO TESTING

In the late 1960's it was determined that cytotoxicity, as expressed as the ED50 (dose that was toxic to 50% of the cells) for testing in the 9KB5 (human epidermoid carcinoma of the mouth) was not predictive of clinical efficacy. Therefore, testing in this system was limited to the testing of natural product fractions, where it was useful in following the isolation of a cytotoxic fraction from either a water, alcohol, or chloroform extract that was shown to be cytotoxic. When the cytotoxic moiety was purified, this purified material was then tested for efficacy in vivo. The protocol for the 9KB5 testing is published in Cancer Chemotherapy Reports, Part 3, Volume 3, No. 2, page 17, 1972. During the period of time that this system was used in the program, the ED50 value for "activity" was either raised or lowered, depending upon the yield.

In 1978, in vitro assays using the L1210 and P388 murine tumors were established. These systems were also used for following the isolation of the "cytotoxic" moiety of natural product extracts. It was cost efficient to use an in vitro assay, in place of an in vivo assay, as well as having a faster "turn-around-time" assay for the natural product chemists. The protocols for these systems were not published, but appear in detail on this Web Page. Chang liver cells (9CH5) were grown in culture, treated on days 1-3 or 1-4, cytotoxicity determined on day 4 or 5 and cytotoxicity expressed as ED50.

HUMAN TUMORS GROWN IN OTHER THAN "NUDE" HOSTS

Details for this testing were published in the appendix of the 1972 publication and appears on this Web Page.

HUMAN TUMOR XENOGRAFT TESTING BASED UPON IN VITRO TESTING (1986-)

The table that appears elsewhere on this web page lists the human tumors in the Program, their data processing code, days to reach 80-60 mg, doubling time at the 200—400 mg weight, and the treatment schedule. Treatment was initiated at the staging day. That is, when the tumors reached a specified weight range and the experiment was initiated. The treatment regimen was based upon the doubling time of the tumor. However, this regimen might be changed (for example, from Q4Dx3 to QD1-5) dependent upon pharmacokinetics and other factors. Thus, the regimen listed under "Treatment Schedule" is the initial regimen. Changes were made to the general protocol as information was accumulated. Evaluation of the efficacy of a tested material was based upon the T/C Percentage (weight of tumor or survival time of the treated mice divided by that of the untreated control) and the effect of the material upon the growth of the tumor (delay). As a general rule, a % T/C value equal to or less than 42 was considered indicative of some degree of efficacy in tumor models where the evaluation was based upon mean/median tumor weight. In all reviews for efficacy, the toxicity to the host, by the agent being tested, was considered.

PREPARATION OF MATERIAL FOR TESTING

A small amount of the material to be tested was placed in a test tube and a few drops of saline were added. If the material went into solution, saline was the vehicle. When the material floated on the surface of the saline, a few drops of Tween 80 was added to decrease surface tension. If the material formed a suitable suspension, two (2) drops of Tween 80 were added to saline when preparing the dosage formulation. Where poor suspensions were obtained, e.g. rapid precipitation of the suspension, Klucel was used as the vehicle. Use of a sonicator was discouraged due to the fear that the heat at the point of the probe might denature or alter the chemical structure of the material to be tested. Formulation was performed by placing the test material in a tissue homogenizer (grinder), adding the appropriate vehicle, and moving the homogenizer tube approximately two (2) times up and down the pestle.

Listed below are outlines and detailed protocols of tumor systems that are/were employed in the program. For some of the tumor systems listed in outline format, detailed protocols are printed in the publications noted above.

PROTOCOLS

The Following protocols were either a) published in *Cancer Chemotherapy Reports* #25, December 1962, and utilized prior to 1972, b) instructions given to the Screening Laboratories and never published, or c) protocols under development and never used as Program Testing changed. No protocol was formally written for the Madison 109 Lung Carcinoma as this tumor model was not routinely used. The methodology for testing in the Madison 109 Tumor system can be found in *Cancer Treatment Reports vol. 61, No. 8: 1459-1470, November 1977.* In brief, a tumor brei in a concentration of 5 X 10(5) cells in 0.1 ml of Hanks' balanced salt solution was inoculated into the right hind leg of male BALB/c mice. Ip treatment commenced 24 hours after tumor implant. Both median survival time and tumor size were recorded and lung metastases was determined by the method of Wexler (Wexler H. *Journal of the National Cancer Institute* 36: 641-645, 1966.